**1982-Pos Board B712**

**An Improved Surface Passivation Method for Single-Molecule Studies**

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We report a surface passivation method for single-molecule studies that can decrease non-specific binding of biomolecules by at least 10-fold as compared to the widely used polyethylene glycol (PEG) surface. As demonstrated for a variety of biological systems, the new surface does not perturb the behavior and activities of biomolecules. Reduction in preparation time and cost is another major advantage. The reported approach can replace the PEG protocol and expand the reach of the powerful single-molecule experimental tools.

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**Single-Molecule Analysis of the Rotation of F1-ATPase under High Hydrostatic Pressure**

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$F_1$-ATPase is the water-soluble part of ATP synthase and is an ATP-driven rotary molecular motor that rotates the rotary shaft against the surrounding stator ring, hydrolyzing ATP. Although the mechanochemical coupling mechanism of $F_1$-ATPase has been well studied, the molecular details of individual reaction steps remain unclear. In this study, we conducted a single-molecule rotation assay of $F_1$ from thermophilic bacteria under various pressures from 0.1 to 140 MPa [1, 2]. Even at 140 MPa, $F_1$ actively rotated with regular 120° steps in a counterclockwise direction, showing high conformational stability and retention of native properties. Rotational torque was also not affected. However, high hydrostatic pressure induced a distinct intervening pause at the ATP-binding angles during continuous rotation. The pause was observed under both ATP-limiting and ATP-saturating conditions, suggesting that $F_1$ has two pressure-sensitive reactions, one of which is evidently ATP binding. The rotation assay using a mutant $F_1$(ßE190D) suggested that the other pressure-sensitive reaction occurs at the same angle at which ATP binding occurs. The activation volumes were determined from the pressure dependence of the rate constants to be $+100$ Å$^3$ and $+88$ Å$^3$ for ATP binding and the other pressure-sensitive reaction, respectively. These results are discussed in relation to recent single-molecule studies of $F_1$ and pressure-induced protein unfolding.

[1] Nishiyama M. and Y. Sowa. 2012. Microscopic Analysis of Bacterial Motility at High Pressure. *Biophys. J.***102**:1872-1880.
[2] Okuno D., M. Nishiyama, and H. Noji. 2013. Single-Molecule Analysis of the Rotation of $F_1$-ATPase under High Hydrostatic Pressure. *Biophys. J.***105**:1635-1642.

**1984-Pos Board B714**

**Fabrication and Surface Functionalization of Highly Birefringent Particles for Optical Torque Wrench**

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Conventional optical tweezers are limited by the ability to apply only translational manipulation to probe the diverse biological systems. The recent extension of optical tweezers, i.e., the optical torque wrench (OTW), allows the direct application and measurement of torque. The OTW provides a platform to measure rotational dynamics of biomolecules and motors including the torque-dependence of DNA or DNA-protein interactions, and of powerful machines such as F0F1-ATP-synthase or bacterial flagellar motor.

The applicable torque of the OTW is largely dependent on the birefringence of the trapped particle. Quartz (SiO2) is widely used due to its facile fabrication and stability in biological environments. However, the birefringence of quartz is limited to fully investigate the torque-speed relationships of diverse biological systems so we explored more highly birefringent crystals such as rutile (TiO2) and vanadate (YVO4). Developing novel fabrication protocol is required because the particle fabrication from these alternative crystals is not as straightforward as quartz. For example, Cr was used as a mask for dry etching cylinders from rutile with SF6/CH4 processing gases and it resulted in etching selectivity of 1:28 which is higher than electron beam resist mask with selectivity of 1:1.3.

To promote the selective attachment of the particle to target biological system, a submicron-sized protrusion can be fabricated on one end of a cylindrical particle and functionalized with specific ligand biomolecules. Alkoxysilane based surface crosslinkers, such as APTES, that are commonly used to bind biomolecules to solid supports, tend to polymerize and result in inhomogeneous surface coatings. To obtain uniform, dense, and reproducible surface coating for nanoscale structures, we explored the use of cyclic aza-silanes as alternative crosslinkers for biomolecule attachment.

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**Combination of Optical Tweezers with Nanocapillaries as System for Estimation of DNA/Ligand Interactions**

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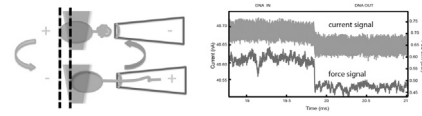
Nanopores are currently widely used for single molecule-studies of biopolymers. However, the velocity of translocation of molecules through nanopores is extremely fast, which is a drawback for detailed investigation of translocating molecules. One of the solutions of this problem is combination of nanopore with optical tweezers. This system allows stalling a DNA molecule inside a nanopore. However, to combine these to systems is not simple due to not trivial design of sample cell, high noise in current signal and necessity of expensive machines for nanopore production. Instead we showed that combination of optical tweezers with glass nanocapillaries, produced simply by laser puller, allows to create the system with many advantages in comparison to the previous one. In this system a bead, containing DNA molecules is brought towards a nanocapillary and after switching on electrical field the current drop and change of force, acting on the trapped bead, are observed due to the DNA translocation. On the next step DNA ligand will be added and DNA/ligand complex will be pulled out of the capillary. The additional current drop can estimate the position of the ligand during translocation.




**1986-Pos Board B716**

**Magnetization Properties of Superparamagnetic Beads**

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Magnetic beads are frequently used in molecular biology and biophysics. In particular, nanometer- to micrometer-sized magnetic beads are a key component in magnetic tweezers and enable the application of precisely calibrated forces and torques to individual molecules of interest. Interestingly, despite the extensive use of magnetic beads, the origin of some of their apparent magnetic properties remains controversial. While the forces exerted on the particles in magnetic tweezers are well understood and have been quantitatively modeled from first principles (Lipfert et al., Biophys J., 2009), the torques are less well understood. Application of torque in magnetic tweezers requires the magnetization of the beads to exhibit some amount of anisotropy, the exact mechanism, however, remains poorly understood. Two models have been put forward: one relies on a small ferromagnetic component in addition to a larger paramagnetic component, while the other model postulates an 'easy' magnetization axis. We have performed two separate experiments to address this issue. First, we used direct angle tracking (Lipfert et al., Rev. Sci. Instrum., 2011) of DNA-tethered beads held in conventional magnetic tweezers to determine the magnetic field dependence of the rotational trap stiffness. The results are well described by a model proposed by Pavone and coworkers (Normanno et al., PRA, 2004) and argue in favor of a weak axis model. Second, we probed beads tethered to rotating flagellar motors of fixed E. coli cells to determine the field-dependent angular speed. Again, the studies suggest that the beads possess an 'easy' magnetization axis. Further insight and understanding of the intrinsic magnetization properties is desirable to improve the sensitivity of single-molecule magnetic tweezers and will be important for a quantitative understanding and optimization of the torque exerted in magnetic tweezers.

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**Probing the Kinetics of a Model Helicase-Nuclease with a Temperature-Controlled Magnetic Tweezers**

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Motor protein activities such as ATP hydrolysis and translocation are temperature-dependent. Their characteristic physicochemical parameters, e.g. the ATP coupling efficiency and the translocation step size, can be